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THE GENETICS OF ARTEMIA SALINA. IV. HYBRIDIZATION OF WILD POPULATIONS WITH MUTANT STOCKS[1]

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The brine shrimp *Artemia salina* is a crustacean (Order Anostraca) which lives in the saline lakes and coastal salterns of five continents. (*Salterns* are ponds where sea water is concentrated by solar evaporation for the commercial production of sodium chloride.) Some populations are bisexual whereas others consist only of females which reproduce parthenogenetically. Some populations are diploid whereas others have been reported to be triploid, tetraploid, pentaploid, or octaploid on the basis of cytological studies. The morphological and cytological studies of *Artemia* populations have been reviewed by Stella (1933), Goldschmidt (1952), Barigozzi (1957), Dutrieu (1960), Gilchrist (1960), and Stefani (1961). The author knows of only two papers in which hybridization studies were mentioned. Gilchrist (1960, page 233) stated that North African shrimp and Californian shrimp would not interbreed. Barigozzi and Tosi (1959) mated Great Salt Lake shrimp to a diploid stock which evidently came from the Gulf of California.

The purpose of the present study was to determine whether nine wild populations of *Artemia* were reproductively isolated from one another. Evidence of reproductive isolation would be: (1) inability of the two populations to live in the same medium (habitat isolation), (2) failure of the male to clasp the female (ethological isolation), (3) failure to produce a viable F_1 (due to mechanical isolation, gametic or zygote mortality, or hybrid inviability), or (4) hybrid sterility (absence of an F_2 or production of a deficient F_2). These isolating mechanisms have been defined and discussed by Mayr (1963, pp. 91 to 109).

This paper describes a series of hybridizations in which wild-type shrimp were out-crossed either to wild shrimp from different localities or to inbred stocks homozygous for recessive mutant genes. It was found that the shrimp from Mono Lake and from Sète were reproductively isolated from each other and from the other seven populations. However, there was no barrier to gene exchange among

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the other populations which therefore represent seven geographical races within a single species.

Materials and Methods

Culture technique

The glassware, genetic techniques, and standard feeding schedule have been described in an earlier paper (Bowen, 1962). Mono Lake shrimp were cultured in water collected from the lake in May (sp. gr. of 1.046). The other wild races, the mutant stocks, and the progeny of experimental matings were cultured in the *standard medium* (50 grams of NaCl per liter of filtered sea water). The females are viviparous when cultured in this medium and fed yeast according to the standard feeding schedule. They do not produce dormant cysts unless the medium is modified by the addition of monosodium iron (ferric) ethylenediaminetetraacetic acid.

Females were isolated at least two weeks before they were mated to males from another race or stock. Genetic experiments have shown that females do not store sperm from one reproductive cycle to the next (Bowen, 1962). All matings described in this paper were single-pair matings in shell vials containing 5 ml. of the standard medium. Every week, the matings were checked and if nauplii were present, they were transferred to other shell vials (2–3 nauplii per vial). All shrimp were routinely examined without anesthetization under a dissecting microscope (7 ×) once per week.

Source of the wild populations

Sète. Cysts of this parthenogenetic race from salterns on the Mediterranean at Sète, France, were obtained through the courtesy of Dr. Barbara M. Gilchrist. The Sète stock is descended from a single female hatched from a cyst in 1961.

San José Island. This stock is descended from ten adults collected by Jean Hanson from the salterns on this island in the Gulf of California near La Paz, Baja California Sur, Mexico.

Little Manitou Lake. Cysts from this lake in Saskatchewan, Canada were obtained through the courtesy of Mr. R. P. Dempster, President of the San Francisco Aquarium Society.

Quemado. Cysts were collected by Mr. Thomas D. Foster from the salt lake near Quemado, New Mexico, U. S. A.

Great Salt Lake. The shrimp used in the racial crosses came from four samples of cysts collected in different years from this lake in Utah, U. S. A. Two collections were made by Mr. C. C. Sanders, one by Mr. M. Rakowicz, and one by Dr. J. S. Hensill.

San Diego. Cysts were collected by Mr. D. M. Miller, an executive of Western Salt Company, from salterns on South San Diego Bay, California, U. S. A.

Moss Landing. Our stock is descended from two adults collected by Miss June Akiyoshi from salterns on Monterey Bay, California, U. S. A.

San Francisco. The shrimp used in the racial crosses were derived from four samples of cysts collected in different years from salterns of the Leslie Salt Company at the south end of San Francisco Bay, California, U. S. A. Three collec-

tions were made by the author or her students and one collection was obtained from Mr. M. Rakowicz, President of Brine Shrimp Sales Co.

Mono Lake. Adults from this lake in California, U. S. A., were collected by the author in 1961 and by Dr. Joel Gustafson in 1963.

Origin of the mutant stocks

Stock #1. This inbred line is descended from shrimp from Great Salt Lake and is homozygous for the recessive gene, *r*, which determines red eyes (Bowen, 1962). This stock has been carried through more than 40 generations in the laboratory.

Stock #9. The origin of this stock has been described earlier (Bowen, 1963). It is derived from both the San Francisco and Great Salt Lake populations and consists of white-eyed males and pigmented-eyed (red or black) females. White eyes is determined by a recessive mutant gene, *w*. The white locus is located on the homologous portion of the sex chromosomes. In the #9 stock, crossing over between the white locus and the sex locus is suppressed. Therefore, this stock breeds true for X^wX^w males and X^wY^+ females.

Results

1. *The Mono Lake population*

Mono Lake shrimp were cultured through three generations in the laboratory in Mono Lake water. However, in each of four independent experiments, ten pairs of Mono Lake shrimp died within one week after transfer to the standard culture medium. Similar attempts to culture Great Salt Lake and San Francisco shrimp in water from Mono Lake were unsuccessful. In another series of experiments, shrimp from Mono Lake and from San Francisco salterns were placed in solutions which combined varying amounts of standard medium, Mono Lake water, or saltern water. Every combination supported one population; no combination was satisfactory for rearing both populations. Therefore, hybridization experiments could not be carried out with shrimp from Mono Lake.

2. *Attempts to mate mutant males with parthenogenetic females*

The Sète stock has been carried through 15 generations in this laboratory. More than 1000 offspring have been reared to maturity; all were parthenogenetic females.

The reproductive cycle of the *Artemia* female has been described by many authors. The viviparous female expels from the uterus a first brood of nauplii. The next brood of eggs passes from the ovaries into the oviducts where they remain for about one day. At this time they are in metaphase of the first meiotic division. They pass next into the uterus where segmentation occurs. Finally they are expelled into the culture medium as the second brood of nauplii. In the Sète population, the eggs develop without fertilization. In the Utah bisexual population, copulation must occur at the time the eggs are in the oviduct if fertilization is to occur (Bowen, 1962).

A series of single-pair matings was made: each consisted of a wild-type (black-eyed) Sète female and either a red-eyed or white-eyed male (from stock #1 or #9). Daily observations were made of clasping and attempted copulation in relation to the female reproductive cycle. Forty-one broods of nauplii were obtained; all matured into wild-type females. Eleven of these broods came from matings in which the male was seen to be clasping and attempting to copulate at the time the eggs were in the oviducts. These 11 broods consisted of 112 wild-type female shrimp. Although they were reared in the absence of males, they produced a second generation of offspring of which 176 shrimp were classified and found to be wild-type females.

If fertilization had occurred in the original matings, one would expect that the sperm would affect development of the parthenogenetic egg in such a way as to produce an abnormal F_1 (consisting of intersexes, or sterile triploids, or shrimp bearing patches of mutant tissue of androgenetic origin). However, all of the first and second generation progeny were wild-type parthenogenetic females. We can conclude that although males will clasp and attempt to copulate with Sète females, the sperm do not affect the development of the Sète embryo.

3. *Differences in morphology of wild populations*

When reared under identical environmental conditions, some wild-type populations of *Artemia* are morphologically distinguishable. Gilchrist (1960) has shown that if shrimp of the same total length are compared, females of the California (San Diego) stock have a shorter and broader abdomen than females of the Algerian stock. The two populations were reared under identical conditions in order to rule out the effect of salinity on body form (reviewed by Gilchrist, 1959, 1960). The present author has also observed quantitative differences in body dimensions in certain races grown for two or more generations under standard

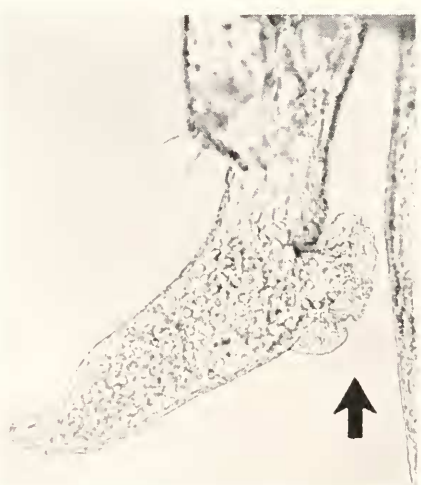

FIGURE 1. Antenna of wild-type female from Quemado, New Mexico. The three-lobed projection (arrow) on the posterior surface is present in females of this race only. Photograph of living animal (lateral aspect, 60×).

TABLE I

Presence of antennal projection

	Females number with projection / total number examined	Males number with projection / total number examined
Great Salt Lake	0/10	0/10
San Francisco	0/10	0/10
Quemado	10/10	0/10
F_1 from matings of Quemado ♂♂ to San Francisco ♀♀	11/12	0/7
Progeny of F_1 ♂♂ × San Francisco ♀♀	24/41	0/23
Progeny of F_1 ♀♀ × San Francisco ♂♂	8/21	0/13

laboratory conditions. Only one qualitative morphological difference has been found: an antennal structure present in females from the Quemado race but absent in all other wild shrimp. This structure and its mode of inheritance will now be described.

The head of the brine shrimp bears a pair of slender unjointed *antennules* and a larger pair of *antennae*. The antennae show sexual dimorphism, being greatly enlarged and modified for clasping in the male. In the Quemado race, the females develop a *projection* on the posterior surface of the antenna, located about one-third of the distance from the distal tip. In young sexually mature females (four weeks old), the projection is a small spike. In older females, the projection has a broader base and terminates in two or three lumps (Fig. 1). No similar structure could be found on the antennae of females from Utah or San Francisco or of males from Great Salt Lake, San Francisco, or Quemado (Table I). This trait was of interest because of the possibility that it might be determined by genes on the non-homologous portion of the Y chromosome. The female is the heterogametic sex in *Artemia* and has the XY chromosome constitution (Bowen, 1963).

Four matings of Quemado males to San Francisco females were fertile. Some F_1 progeny from each mating were backcrossed to San Francisco shrimp. Because of the time required to examine the antennae under high power magnification (160 ×), only a small sample of the F_1 and backcross progeny were examined for the presence of the projection. More females were examined because it was evident that males could not have the projection (see the data in the last three rows in Table I). Note that most of the F_1 females have the projection. This indicates that the Quemado males can transmit this trait to their daughters. Both male and female F_1 hybrids can transmit the trait to their daughters. We may conclude that this projection is determined by autosomal dominant genes at one or more loci. These genes are sex-limited; that is, they are present in both sexes but have no effect upon the phenotype of the male.

In the preceding discussion, the author has assumed that the projection on the Quemado female antenna is not homologous with the *frontal knob*, a disc-like projection on the basal segment of the antennae of males from all *Artemia* populations. However, even if these two structures were homologous, we would again conclude that the projection-knob trait is governed by autosomal loci.

4. *Hybridization among seven bisexual North American populations*

The seven populations which were successfully hybridized were from San José Island (Mexico), Little Manitou Lake (Canada), and from five localities in the U.S.A.: Great Salt Lake, Quemado, San Francisco, Moss Landing, and San Diego. Evidence has been presented above (Table I) that matings of Quemado males to San Francisco females gave progeny which were fertile when backcrossed to San Francisco shrimp. In a second series of matings, wild-type shrimp were mated to wild shrimp from other localities and the progeny were mated *inter se* to produce an F_2 (Table II). In a third series of matings, reciprocal crosses were made of each of the seven populations to shrimp from stock #1 which is homozygous for the recessive mutant gene for red eyes. The F_1 progeny were mated *inter se* to produce an F_2 (Table III). The experiments summarized in Tables I and III provide a rigorous proof of hybridization because the recovery of the genetic markers (antennal projection and red eyes, respectively) rules out the possibility of parthenogenetic or pseudogamic reproduction or experimental errors such as the use of non-virgin females in the outcrosses.

The experiments summarized in Tables II and III were designed in such a way that an F_1 and F_2 would be obtained from every fertile parental pair. For example, in Table III nine matings were set up in which males from the mutant stock were mated to females hatched from cysts collected from Great Salt Lake. Each of the eight fertile pairs produced at least one F_1 brood which when mated *inter se* gave rise to a normal F_2 brood.

In Tables II and III, the source of the wild parents (either cysts collected from the natural habitat or inbred laboratory stocks) is stated to enable the reader to determine the number of independent genotypes which were sampled from each

TABLE II

Hybridization of six wild populations of brine shrimp

Parental cross Male × Female	Source of parents	Parental crosses fertile pairs / total matings	Number of F_1 hybrids reared to maturity	Normal F_2 progeny obtained from each fertile parental pair
Gt. Salt Lake × Gt. Salt Lake (control)	cysts	6/8	116	
San Francisco × San Francisco (control)	cysts	9/13	176	
San Francisco × Gt. Salt Lake	cysts	2/6	83	yes
Gt. Salt Lake × San Francisco	cysts	1/3	113	yes
Little Manitou × Quemado	cysts	2/2	130	yes
Quemado × Little Manitou	cysts	1/2	17	yes
San José Is. × Gt. Salt Lake	inbred stocks	3/3	110	yes
San José Is. × San Francisco	inbred stocks	2/2	25	yes
San Francisco × Moss Landing	inbred stocks	1/1	5	yes
Little Manitou × San Francisco	cysts	1/1	28	yes

TABLE III

Results of mating seven races of wild brine shrimp to stock #1 which is homozygous for the recessive gene for red eyes

Parental cross Male × Female	Source of wild parents	Parental crosses fertile pairs / total matings	Number of F_1 hybrids reared to maturity (wild phenotype)	Number of F_2 progeny reared to maturity	Red-eyed shrimp found in F_2 from every fertile parental pair
Gt. Salt Lake × stock #1	cysts	5/8	283	94	yes
stock #1 × Gt. Salt Lake	cysts	8/9	606	421	yes
San Francisco × stock #1	cysts	3/4	342	117	yes
stock #1 × San Francisco	cyst	1/1	61	227	yes
Quemado × stock #1	cysts	6/7	329	93	yes
stock #1 × Quemado	cysts	3/4	291	261	yes
Little Manitou × stock #1	inbred stock	4/5	130	63	yes
stock #1 × Little Manitou	inbred stock	2/5	190	63	yes
Moss Landing × stock #1	inbred stock	1/1	6	9	yes
stock #1 × Moss Landing	inbred stock	1/1	14	67	yes
San Diego × stock #1	cysts	2/3	68	61	yes
stock #1 × San Diego	cysts	1/3	9	8	yes
San José Is. × stock #1	inbred stock	4/4	155	36	yes
stock #1 × San José Is.	inbred stock	1/1	16	14	yes

population. The next column in both tables gives the fraction of successful (fertile) matings. This is primarily an indication of the efficiency of the laboratory technique. That is, the failure of a mating may be due to the death or poor health of one parent rather than to reproductive isolation of a genetic nature. Evidence supporting this interpretation comes from the observation that only 9/13 of the control matings, San Francisco ♂♂ × San Francisco ♀♀, were successful (Table II). In another study of 46 matings of stock #1 ♂♂ × stock #1 ♀♀, at the end of a two-week period only 31 females were alive and of these only 16 had produced progeny.

Although a few F_1 nauplii from each hybridization were examined carefully under the high-power microscope (120 ×), no abnormal hybrids were found of the type described by Barigozzi and Tosi (1959). They stated that when Great Salt Lake *Artemia* were crossed to a diploid race, the F_1 offspring were triploids of abnormal appearance: stunted body and enlarged lateral eyes and limbs.

The experiments summarized in Tables II and III were designed to sample the F_1 and F_2 generations from the greatest number of parental crosses. In order to speed the sampling process, many broods were discarded before the last shrimp in the brood reached the stage of sexual differentiation. (There is great variation in growth rates, even in highly inbred lines of *Artemia*.) For this reason, sex ratios and genetic segregation data are not given in either table. However, aberrant red eye segregation ratios and aberrant sex ratios were not noted. At weekly in-

tervals, the number alive in each brood was recorded. The viability of the hybrids did not differ significantly from that of the controls (San Francisco wild-type nauplii). Forty-nine per cent (220/450) of the San Francisco shrimp were alive at the end of three weeks.

Three F_2 broods from parental crosses of stock #1 × Great Salt Lake and four F_2 broods from crosses of stock #1 × San Francisco were reared to maturity and their eyes were examined at 4-day intervals. In order to classify eye color correctly, each shrimp must be isolated and examined throughout development because some heterozygotes have ruby eyes for a brief period (Bowen, 1962). The combined values from the seven broods were: 34 with red eyes and 115 with black eyes or 34/149 (23%) with *r/r* genotype. This is in good agreement with the value of 25% expected in the F_2 progeny from a cross of two parental diploids.

The sex-ratio data in Table IV are taken from some of the experiments summarized in Tables II and III and from additional matings of wild-type populations with mutant stocks. The ratios do not show significant deviations from the expected value of 50% males.

TABLE IV

Sex ratios in F_1 progeny of crosses of wild-type brine shrimp to mutant stocks

Parental cross Male × Female	F_1 hybrids		Per cent males
	Males	Females	
Gt. Salt Lake × mutant stock	178	166	52%
mutant stock × Gt. Salt Lake	120	132	48
San Francisco × mutant stock	148	140	51
mutant stock × San Francisco	196	200	50
Quemado × mutant stock	106	119	47
mutant stock × Quemado	130	144	47
Total	878	901	49

DISCUSSION

The parthenogenetic population

When males from mutant stocks were mated to parthenogenetic females from Sète, hybrid offspring were not obtained (see part 2). These negative results suggest that males cannot introduce genes into the parthenogenetic population. The Sète population consists of a cluster of clones, each of which is reproductively isolated from every other clone and from males of other localities. Each Sète female is a genetic isolate.

The Mono Lake population

Hybridization experiments could not be made with the shrimp from Mono Lake because they have different physiological requirements; they die when trans-

ferred into media in which the other populations were cultured (see part 1). These negative results suggest that the Mono Lake bisexual population cannot exchange genes with the other seven bisexual populations. It is reproductively isolated due to habitat selection. Because it is morphologically similar to other populations of *Artemia* (see part 3), it represents a sibling species.

The seven geographical races

The other seven bisexual populations (from San José, Little Manitou, Great Salt Lake, Quemado, San Francisco, Moss Landing, and San Diego) are not reproductively isolated and therefore represent geographical races within a single species. Evidence for this is the fact that they produce hybrids with normal viability and fertility. Although the wild populations were not crossed in every possible combination, it was evident that shrimp from the ends of the Pacific Coast distribution of salterns (San Francisco and San José Island) were cross-fertile with each other and with the Great Salt Lake race. Similarly, the shrimp from the most northern and southern inland lakes (Little Manitou and Quemado) were cross-fertile with each other and with the San Francisco race (Table II).

In a second series of matings, reciprocal crosses were made of each race with shrimp from a mutant stock homozygous for the recessive gene for red eyes (Table III). The F_1 progeny had normal morphology, viability, and fertility and the mutant phenotype was seen again in the F_2. The sex ratios in the F_1 generation of each cross did not show significant deviation from the expected value of 50% males (part 4 and Table IV).

There is no intrinsic barrier to gene exchange among these races. Genes from one race might be introduced into another if cysts were carried into a new habitat on the legs or in the digestive tracts of migrating birds. However, geographical isolation has been sufficiently effective to bring about the divergence of the Quemado population in regard to the morphology of the antenna (described in part 3).

Ploidy of the seven bisexual populations

The seven races listed in Tables II and III must be at the same level of ploidy; *i.e.*, all seven must be diploid or all seven must be tetraploid. For, if a diploid were crossed to a tetraploid, the hybrid offspring certainly would be sterile triploids, due to irregular meiosis, and possibly the sex ratio would be altered.

Nakanishi, Okigaki, Kato and Iwasaki (1963) made chromosome counts on somatic cells of nauplii hatched from cysts obtained from the San Francisco Aquarium Society. Cysts distributed under the Society name are collected by Brine Shrimp Sales Company of Hayward, California, from the salterns of the Leslie Salt Company on San Francisco Bay (personal communication from Mr. James A. Mason, General Manager of Brine Shrimp Sales Company). Nakanishi *et al.* reported that the chromosome number ranged from 16 to 48 but there was a distinct peak formed by cells showing 42 chromosomes. If the San Francisco race is a diploid ($2n = 42$), we must conclude that the six races which produce fertile hybrids when crossed to this race are also diploid. This line of reasoning indicates that the seven cross-fertile races are diploid.

This conclusion is not in agreement with the statement of Metalli, Ballardin and Barigozzi (1961) that the race from San Francisco Bay was "predominantly diploid" whereas the race from Great Salt Lake was "predominantly tetraploid" (pages 410 and 417 of their 1961 paper). Barigozzi and Tosi based their decision that the Utah shrimp were tetraploid ($4n = 84$) on cytological studies and on the results of a hybridization experiment. Seven matings were made of Great Salt Lake shrimp to animals from a diploid stock. The hybrid progeny were abnormal in appearance and died before reaching maturity (Barigozzi and Tosi, 1959). It is difficult to evaluate these negative results because the authors did not state their culture method or give an estimate of the viability of non-hybrid nauplii. Somatic chromosome counts on the abnormal hybrids (presumed triploids) gave values in the range from 32 to 63. Additional evidence for tetraploidy came from chromosome counts on male germ cells, nauplius somatic cells, and primary oocytes of the Great Salt Lake race (Barigozzi and Tosi, 1957, 1959). When oocytes of Utah females were examined, the metaphase-I plates consisted in some cases of "42 isolated tetrads" while in other plates these tetrads were "ordered two by two, corresponding to 21 tetravalent bodies" (page 3 of their 1959 paper). The genetic experiments reported in the present paper suggest that the 21 "tetravalent" elements may have been 21 tetrads, *i.e.*, 21 bivalents. In the cytological studies of parthenogenetic *Artemia*, there have been disagreements among several authors in regard to the interpretation of the elements seen on the metaphase-I spindle of several races (reviewed by Goldschmidt, 1952). For example, the Kalia race was first reported to be a decaploid because the 107–109 elements were interpreted as tetrads (bivalents). In a later study, they were interpreted as univalents and the race was reported to be pentaploid (Haas and Goldschmidt, 1946; Goldschmidt, 1952).

Several explanations might be advanced for this lack of agreement between the conclusions in the present paper and those of Dr. Barigozzi and his co-workers in regard to the ploidy of the Utah shrimp. One possibility might be the use of wild shrimp of mistaken origin. However, in the studies reported here, the shrimp from Great Salt Lake, Utah, were hatched from four samples of cysts which were collected and handled independently. Readers who are acquainted with the earlier literature of *Artemia* might wonder whether the progeny obtained from outcrossed Utah females might be explained by parthenogenesis in the Utah population. (Many textbooks state that the Utah race is parthenogenetic.) However, parthenogenesis has never been observed in the seven North American races maintained in this laboratory (those listed in Table III). Extensive genetic tests for pseudogamic and parthenogenetic reproduction were conducted on Utah shrimp without positive results (Bowen, 1962). There are two more probable explanations of the disagreement. First, some cysts from Great Salt Lake may indeed be tetraploid. These may have given rise to the stock used by Dr. Barigozzi. In that case, we would conclude that Great Salt Lake contains two reproductively isolated sympatric populations: one diploid and one tetraploid. Such a situation would not be stable for long since every $4n \times 2n$ mating would result in the elimination of an equal number of gametes from each population. This process would eventually eliminate the population which had the smaller initial numbers. Another possibility is that the Great Salt Lake race is entirely diploid. In that case, the inviable progeny

obtained in the hybridization experiments of Barigozzi and Tosi (1959) might have resulted from the chance selection of seven females which would have given birth to inviable progeny regardless of the origin of their mates. Some matings consistently produce thin-shelled eggs which soon decompose or broods of nauplii which die before reaching sexual maturity (Bowen, 1962, p. 27, and Part 4 of this paper).

Evidence has been presented that the Great Salt Lake race of *Artemia* is cross-fertile with six other North American races: San José Island, San Diego, Moss Landing, San Francisco, Little Manitou, and Quemado. Sixteen shrimp, each hatched from an encysted blastula collected from the shores of Great Salt Lake, were crossed to San Francisco shrimp or to a mutant laboratory stock known to be cross-fertile with the other six races (Tables II and III). The F_1 males and females from all sixteen matings were normal in appearance and fertile. In thirteen of these matings, a genetic marker was used and the recessive mutant trait was recovered in the F_2 generation (Table III). Genetic segregation ratios were recorded from some of these matings and the data did not deviate significantly from the values expected in the F_2 of two parental diploids (see part 4). The F_1 sex ratios did not deviate significantly from the expected 1:1 ratio of males to females (Table IV).

Summary

Brine shrimp were collected from eight bisexual populations in North America and from one parthenogenetic population in Sète, France.

1. Males from mutant bisexual stocks were mated to wild-type Sète females. The progeny were wild-type females which parthenogenetically produced a second all-female generation. Although males will clasp and attempt to copulate with Sète females, their sperm do not affect the development of the parthenogenetic egg.

2. Hybridization experiments could not be carried out with the bisexual shrimp from Mono Lake because they died when transferred into the medium in which the other eight populations were cultured. This suggests that the Mono Lake population is reproductively isolated from the others.

3. A projection found only on antennae of females from Quemado, New Mexico, is determined by dominant autosomal genes with sex-limited expression.

4. There was no evidence of reproductive isolation among the *Artemia* from seven North American localities: San Francisco, Great Salt Lake, San Diego, Moss Landing, San José Island, Little Manitou, and Quemado. Reciprocal crosses were made of shrimp from each of these seven populations with shrimp from a mutant stock which was homozygous for the recessive gene for red eyes. In all crosses, the F_1 progeny had normal morphology and viability and the expected 50:50 sex ratio was obtained. The F_1 progeny were fertile and the mutant phenotype was recovered in the F_2 generation. Therefore, these seven populations have no intrinsic barrier to gene exchange and represent geographical races within a single species. Because cytologists agree that the San Francisco race is diploid, it is assumed that the six races with which it is cross-fertile are also diploid. This statement is not in agreement with the conclusion of Barigozzi and Tosi (1959) that the Great Salt Lake *Artemia* are tetraploid.

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